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Urinary Excretion of N-Acetyl-β-*D*-glucosaminidase in Normal and Complicated Pregnancy

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Summary: The urinary excretion of N-acetyl-β-*D*-glucosaminidase activity, a sensitive indicator of renal tubular injury, was monitored during and after pregnancy. During normal pregnancy, enzymuria increased progressively to levels 3–4 times above normal in the third trimester. In diabetic mothers, enzyme excretion followed a similar pattern, but was generally higher than in uncomplicated pregnancies. Also in preeclampsia, enzymuria tended to be higher than in normal pregnancy. Enzyme excretion normalized about a year after normal pregnancies, but remained elevated in diabetic subjects and in patients who had developed preeclampsia. This latter finding indicates that marginal persistent renal damage may occur during preeclampsia.

Introduction

Urinary enzyme determinations have become increasingly useful in the evaluation of renal disorders (1, 2). In particular the lysosomal enzyme N-acetyl-β-*D*-glucosaminidase has been studied extensively (1–5). N-Acetyl-β-*D*-glucosaminidase excretion reflects mainly tubular injury (3–6). However, only little information is available regarding urinary excretion of N-acetyl-β-*D*-glucosaminidase in pregnancy (7). We have therefore investigated urinary N-acetyl-β-*D*-glucosaminidase excretion in normal pregnancy and in pregnant women with preeclampsia and with diabetes mellitus.

Materials and Methods

The investigation involved 12 healthy pregnant women, 20 pregnant patients with diabetes and 20 patients with preeclampsia. Their mean age was 31.5, 27.9 and 31 years (range 24–40, 21–37 and 23–41), respectively. The mean duration of diabetes was 12.3 (range 0 to 27) years. The diabetic patients were extremely carefully supervised during pregnancy with frequent monitoring of blood glucose and HbA_1c levels, and the insulin dosage was adjusted for optimal metabolic control. The diagnosis of preeclampsia was based on the development of hypertension (mean arterial blood pressure > 103 mm Hg) and was frequently accompanied by proteinuria (11 patients) and oedema (5 patients). In 14 cases, these patients were treated with β-blockers. Twenty-one healthy, non-pregnant women aged 20–40 years served as controls.

All patients were examined in the morning, but fasting was not considered necessary for the purpose of this investigation.

N-Acetyl-β-*D*-glucosaminidase (N-acetyl-β-*D*-glucosaminide N-acetylglucosaminohydrolase EC 3.2.1.30) activity was determined using *p*-nitrophenyl-2-acetamido-2-deoxy-β-*D*-glucopyranoside (Koch-Light Labs Ltd, Colnbrook, England) as substrate (8). *p*-Nitrophenol was used as standard. One unit (U) of enzyme activity represents the hydrolysis of 1 μmol substrate per min. Urinary creatinine was measured with a kinetic *Jaffé* method. Glomerular filtration rate was measured by determination of iohexol clearance (9). Urinary albumin and β_2-microglobulin were quantitated by commercial radioimmunoassay kits from Pharmacia, Uppsala, Sweden. Significance of differences were analysed by *Wilcoxon*'s rank order test, and correlations tested with *Spearman*'s rank correlation test.

Results

The urinary excretion of N-acetyl-β-*D*-glucosaminidase in non-pregnant women was 0.17 ± 0.09 kU/mol creatinine (mean ± S.D.). As shown in table 1, the excretion of urinary N-acetyl-β-*D*-glucosaminidase is significantly increased in pregnancy. In the second trimester N-acetyl-β-*D*-glucosaminidase excretion was more than two-fold higher than that recorded in non-pregnant women, and in the third trimester an additional increase ($p < 0.05$) was noted.

Proteases of Retroviruses

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The book "Proteases of Retroviruses" provides initial information in the rapidly developing field of examination of proteolytic enzymes involved in the life cycle of HIV and of the most important oncogenic retroviruses. It summarizes the advance of the knowledge of retroviral proteases achieved most recently in virology, molecular biology and enzymology. It will become and invaluable tool for researchers working in these fields as well as for those interested in the design of new antivirals.

From the Contents

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Tab. 1. Urinary excretion of N-acetyl-β-*D*-glucosaminidase in normal and complicated pregnancy. Data are expressed as kU/mol creatinine and given as mean ± S.D.

	Second trimester	Third trimester	6–18 months postpartum
Normal pregnancy	0.43 ± 0.18** n = 7	0.64 ± 0.27*** n = 10	0.11 ± 0.05$^{n.s.}$ n = 7
Pregnancy with diabetes mellitus	0.53 ± 0.32*** n = 14	0.98 ± 0.80*** n = 16	0.70 ± 0.60*** n = 14
Preeclampsia	–	0.90 ± 0.53*** n = 19	0.30 ± 0.21* n = 10
Non-pregnant controls	0.17 ± 0.09 n = 21		

* $p < 0.05$ compared with non-pregnant controls
** $p < 0.01$ compared with non-pregnant controls
*** $p < 0.001$ compared with non-pregnant controls
n. s. = not significant

Also in the pregnant diabetic women, N-acetyl-β-*D*-glucosaminidase excretion was elevated and increased significantly ($p < 0.05$) during the later stage of pregnancy. There were no significant differences between the normal pregnancy group and the diabetic pregnancy group. In the patients with preeclampsia, urinary N-acetyl-β-*D*-glucosaminidase activities were markedly increased; but again, the difference between these activities and those for subjects with normal pregnancy were not statistically significant.

After normal pregnancies the N-acetyl-β-*D*-glucosaminidase excretion decreased and it had normalized by 6–18 months post partum. However, in the preeclampsia group, N-acetyl-β-*D*-glucosaminidase levels remained slightly raised, and in diabetic subjects urinary N-acetyl-β-*D*-glucosaminidase excretion was markedly elevated 6–18 months after delivery.

No correlation was found between the urinary excretion of N-acetyl-β-*D*-glucosaminidase and the glomerular filtration rate. Marked albuminuria (> 20 mg/l) occurred in 2/12 women with normal pregnancy, in 4/20 diabetic subjects, and in 11/20 patients with preeclampsia. For β_2-microglobulinuria (> 0.3 mg/l), the corresponding frequencies were 5/12, 6/20, and 5/20, respectively. There were no correlations between the presence or level of albuminuria, or the concentration of β_2-microglobulin in urine, and the activity of urinary N-acetyl-β-*D*-glucosaminidase.

Discussion

This study demonstrates that the urinary excretion of N-acetyl-β-*D*-glucosaminidase increases gradually during pregnancy to levels 3–4 times higher than those recorded in non-pregnant women. The mechanisms behind the increased N-acetyl-β-*D*-glucosaminidase excretion are unclear, but since we could find no correlation with the glomerular filtration rate, proteinuria or β_2-microglobulin concentrations in the urine, it seems unlikely that the increased N-acetyl-β-*D*-glucosaminidase excretion in normal pregnancy reflects renal injury. In a previous investigation (10) we showed that N-acetyl-β-*D*-glucosaminidase excretion can be increased solely by fever, without obvious renal affection, to levels similar to those observed in pregnancy. Thus, N-acetyl-β-*D*-glucosaminidase in urine is a very sensitive test, and the increased excretion of N-acetyl-β-*D*-glucosaminidase as well as other enzymes (11, 12) in normal pregnancy probably reflects reversible physiological changes that enhance the rate of enzyme turnover, rather than being due to renal injury. This interpretation is consistent with the normalization of N-acetyl-β-*D*-glucosaminidase excretion after delivery.

In preeclampsia, the rise in N-acetyl-β-*D*-glucosaminidase excretion tended to be more pronounced than in normal pregnancy. Also the levels are much more increased than those reported in subjects with essential hypertension (13). The increased enzyme excretion in women with preeclampsia may reflect renal ischaemia and relative hypertension of pregnancy. Urinary N-acetyl-β-*D*-glucosaminidase excretion did not fully normalize after pregnancy. This finding might indicate that the preeclamptic state results in marginal persistent renal tubular damage. However, the prognostic value of persistent enzymuria for later renal function impairment needs to be determined.

We demonstrated earlier that the urinary excretion of N-acetyl-β-*D*-glucosaminidase is significantly increased in patients with diabetes mellitus (14). In the present study we found no further elevation of N-acetyl-β-*D*-glucosaminidase excretion during pregnancy; rather the levels recorded during the second trimester tended to be lower than those in non-preg-

nant diabetic patients (14). After delivery, N-acetyl-β-*D*-glucosaminidase excretion in the diabetic subjects remained significantly elevated, whereas it returned to its lower, pre-pregnancy value in normal pregnancy. We believe that the tendency towards a lower N-acetyl-β-*D*-glucosaminidase excretion in the diabetic patients during pregnancy can be explained by the improved metabolic control of the disease during pregnancy.

While this manuscript was in preparation, *Goren* et al. (7) presented data demonstrating progressively increased N-acetyl-β-*D*-glucosaminidase excretion during normal pregnancy, and a further increase in subjects developing preeclampsia. Our observations confirm their results, and our novel finding that N-acetyl-β-*D*-glucosaminidase excretion does not fully normalize after pregnancy supports their suggestion that preeclampsia is accompanied by tubular lesions, consonant with arteriolar constriction and microinfarction (7).

Acknowledgement

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References

1. Raab, W. P. (1972) Diagnostic value of urinary enzyme determinations. Clin. Chem. *18*, 5–25.
2. Vanderlinde, R. E. (1981) Urinary enzyme measurements in the diagnosis of renal disorders. Ann. Clin. Lab. Sci. *11*, 189–201.
3. Kunin, C. M., Chesney, R. W., Craig, W. A., England, A. C. & De Angelis, C. (1978) Enzymuria as a marker of renal injury and disease: Studies of N-acetyl-β-*D*-glucosaminidase in the general population and in patients with renal disease. Pediatrics *62*, 751–760.
4. Hultberg, B. & Ravnskov, U. (1981) The excretion of N-acetyl-β-*D*-glucosaminidase in glomerulonephritis. Clin. Nephrol. *15*, 33–38.
5. Wellwood, J. M. Davies D., Leighton, M. & Thompson, A. E. (1978) Urinary N-acetyl-β-*D*-glucosaminidase assay in renal transplant recipients. Transplantation *26*, 396–400.
6. Bourbouze, R., Baumann, F.-C., Bonvalet, J.-P. & Farman, N. (1984) Distribution of N-acetyl-β-*D*-glucosaminidase isoenzymes along the rabbit nephron. Kidney Int. *25*, 636–642.
7. Goren, M. P., Sibai, B. M. & El-Nazar, A. (1987) Increased tubular enzyme excretion in preeclampsia. Am. J. Obstet. Gynecol. *157*, 906–908.
8. Hultberg, B. & Wieslander, I. (1982) Urinary excretion of β-hexosaminidase in patients with vesico-ureteric reflux. Acta Med. Scand. *211*, 257–259.
9. Krutzén, E., Bäck, S. E., Nilsson-Ehle, I. & Nilsson-Ehle, P. (1984) Plasma clearance of a new contrast agent, iohexol: A method for the assessment of glomerular filtration rate. J. Lab. Clin. Med. *104*, 955–961.
10. Sandberg, T., Bergmark, I., Hultberg, B., Jagenburg, R. & Trollfors B. (1986) Diagnostic potential of urinary enzymes and β_2-microglobulin in acute urinary tract infection. Acta Med. Scand. *219*, 489–495.
11. Noble, M. C. B., Landon, M. J. & Davison, J. M. (1977) The excretion of gamma-glutamyl transferase in pregnancy. Br. J. Obstet. Gynaecol. *84*, 522–527.
12. Roberts, A. P., Frampton, J., Karim, S. M. M. & Beard, R. W. (1967) Estimation of beta-glucuronidase activity in urinary-tract infection. N. Engl. J. Med. *276*, 1468–1470.
13. Alderman, M. H., Melcher, L. Drayer, D. E. & Reidenberg, M. M. (1983) Increased excretion of urinary N-acetyl-β-*D*-glucosaminidase in essential hypertension and its decline with antihypertensive therapy. N. Engl. J. Med. *309*, 1213–1217.
14. Hanseus, K., Hultberg, B., Isaksson, A. & Sjöblad, S. (1983) Plasma and urinary β-hexosaminidase in juvenile diabetes mellitus. Acta Paediatr. Scand. *72*, 77–80.

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